IDENTIFICATION OF RAPD MARKERS LINKED TO DROUGHT TOLERANCE BY BULKED SEGREGANT ANALYSIS

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Summary

The aim of this work was to investigate drought tolerance of two new sunflower lines with distinctively different tolerance to stem canker disease, and their F_1 and F_2 generations and to identify RAPD markers linked to drought tolerance by the bulked segregant analysis approach.

Plants were grown in greenhouse and have been watered regularly until the flower bud formation (ϕ=2cm). After that watering was stopped. At full flowering leaf relative water content, leaf water potential, green leaf area, leaf dry weight, plant dry weight, stomatal resistance, leaf soluble and Rubisco protein content were determined in order to produce a drought screening system.

DNA was extracted from leaves and several DNA bulk samples were formed according to the drought tolerance evaluation test. Polymorphism of DNA isolated from parental lines, their hybrid and F_2 bulks was investigated with RAPD markers. Obtained similarities and differences are discussed.

Introduction

Drought is one of the most severe environmental stresses that plants encounter and it affects almost all plant functions, including photosynthesis, growth and development. Several genes have been described that respond to acute dehydration at the transcriptional level in a variety of plant species (Iuchi et al., 1996; Jamaux et al., 1997). Since plants rarely experience this kind of water loss under field conditions, there is little direct evidence for the implication of described genes in drought acclimation, even when drought was imposed on young plants over days (Ouvrard et al., 1996). However, water stress prior to and during flowering has a major influence on the yield of sunflower. It is likely that a genetic component to drought tolerance in sunflower will express itself at this stage of development. The aim of this work was to investigate drought tolerance of two new sunflower lines and their F_1 and F_2 generations and to identify RAPD markers linked to the acclimation to long term drought by the bulked segregant analysis approach.

Materials and Methods

Two NS parental lines, their hybrid and F_2 plants were grown in greenhouse. Plants were irrigated regulary until the phase of flower bud (ϕ=2cm). After that watering was stopped. Several physiological traits likely to be important for drought tolerance were measured at full flowering. Leaf relative water content (RWC), leaf water potential (ψ_{H20}), total leaf area, leaf dry mass, plant dry mass, stomatal conductance, total soluble proteins and Rubisco protein contents were determined as in Pankoviæ et al. (1999).

Genomic DNA was isolated from frozen leaves as in Dellaportha et al. (1983). DNA polymorphism was investigated by the use of decamer primers (UBC Canada, Amersham Pharmacia) in polymerase chain reaction (Sossey-Alaoui et al., 1998). RAPD fragments were separated by electrophoresis on a 2% agarose gel.

Results and Discussion

Water status of fully expanded leaves of two sunflower lines, their hybrid and F_2 plants exposed to long term drought is presented in Figure 1. According to our previous results RWC of 80-90 % and Ψ_{H20} of -0.5 to -1.0 MPa correspond to values measured in fully expanded sunflower leaves under optimal watering in flowering. Long term water deficit decreased RWC to 40% and Ψ_{H20} to -3.0 MPa (Pankoviæ et al., 1999). This is exactly the range of water status parameters measured in the leaves of F_2 plants under long term drought in this experiment. Leaf water status of two examined lines did not differ significantly, while that of their hybrid was significantly higher (Fig. 1.). Dry mass of plants with different leaf RWC

under long term drought is presented in Figure 2. Although leaf RWC of two sunflower lines did not differ significantly, plant dry mass as well as total leaf area and leaf dry weight (not presented) in CMS line were significantly lower. Hybrid plants were expectedly superior while F_2 plants covered a broad range from about 20 to 180 g dry mass per plant.

Figure 1. The effect of drought on leaf water potential and relative water content of sunflower lines CMS (∗) and L-19 (_) and their F_1 (>) and F_2 (э, [, 0) hybrids. Points present individual plants except in the case of CMS and L-19 lines and F_1 hybrid, where each point presents the mean value of 10 plants. Plants 68, 79, 95, 100, 101 and 106 show low RWC and high leaf water potential. Plants 36, 48, 52, 69, 94 and 96 show high RWC and low leaf water potential.

Figures presenting total leaf area, leaf dry weight, stomatal conductance, total soluble and Rubisco protein content, versus RWC under long term drought are not presented here since they have similar data distribution as in Figure 2 (Pankoviæ et al., 1998). As illustrated in Figures 1 and 2, according to measured parameters the same plants are always in the drought sensitive group (plants 68, 79, 95, 100, 101, 106) and drought tolerant group (plants 36, 48, 52, 69, 94, 96). Since the vigor of F_2 plants was different even before water stress was introduced, this could also contribute to different drought tolerance. When investigating F_2 plants it is not possible to avoid this effect by introducing the control plants. Thus two more groups were introduced, plants with similar growth parameters but lower (I) and higher (II) RWC (Fig. 2.).

Figure2. The effect of drought on plant dry mass of sunflower lines CMS (∗) and L-19 (_) and their F_1 (>) and F_2 (э, [, 0) hybrids. Points present individual plants except in the case of CMS and L-19 lines and F_1 hybrid, where each point present the mean value of 10 plants. Plants 68, 79, 95, 100, 101 and 106 show low RWC and low plant dry mass. Plants 36, 48, 52, 69, 94 and 96 show high RWC and high plant dry mass.

DNA was isolated from leaves of parental lines, hybrid and each F_2 plant. Polymorphism of isolated DNAs was examined by RAPD markers. Out of 30 random primers five of them revealed polymorphism between parental lines (Fig. 3). According to the grouping previously explained (Fig. 2) four bulk DNA samples were prepared: drought-sensitive, drought-tolerant, and two groups of similar vigor but with different water status. Primers polymorphic for parental lines were not in the same time polymorphic for DNA bulks. So 50 more primers were screened on DNA bulks, out of wich only one showed polymorphism (figure 4.). RAPD fragment marked with an arrow corresponding to 1kb fragment, appeared in parental line L-19, hybrid and bulk 4. This could be a putative marker for better acclimation to long term drought. The screening of other primers is underway both on described bulks as well as on F_2 bulks from the same cross differently tolerant to *Phomopsis,* since genotypes tolerant to *Phomopsis* are often drought tolerant too.

Intraspecific variations for physilogical drought tolerance parameters such as: transpiration efficiency of dry matter production (Virgona et al., 1990), osmotic adjustment (Chimenti and Hall, 1993), leaf water use efficiency (Plesnièar, 1993) and photosynthetic acclimation (Pankoviæ et al., 1999) in sunflower have been observed. Lacombe et al. (1999) have shown that cDNAs corresponding to some drought induced genes reveal polymorphism between sunflower lines and hybrids. RAPD bulk segregant analysis has so far been succesfully employed in investigation of desease tolerance. Jamaux et al. (1997) have applied similar approach to obtain putative molecular marker for relative water loss of excised leaves and osmotic adjustment. Here we describe the putative RAPD marker for better acclimation of sunflower to long term drought which could be implemented in Marker assisted selection for drought tolerance.

Figure 3. Amplification of RAPD fragments with different primers (1-7) on DNA isolated from sunflower lines, L-19 (I) and CMS (II). Arrows indicate the position of polymorphic RAPD markers obtained with primers 1-5. Primers 6 and 7 are covering the identical part of the sunflower genome. 1Kb indicates the molecular marker standard.

Figure 4. The amplification of RAPD fragments obtained with primer UBC137 and parental lines L-19 (1), CMS (2), their hybrid (3), and from DNA bulks: drought-sensitive (4), drought-tolerant (5) and groups of similar plant vigor but low (6) and high (7) water status. The polymorphic fragment obtained in lines 1, 3 and 7 is marked with an arrow.

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